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TECHNICAL MANUSCRIPT 194

IMMUNOGENIC POTENCY OF PASTEURELLA TULARENSIS CELL WALLS

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IMMUNOGENIC POTENCY OF PASTEURELLA TULARENSIS CELL WALLS

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ABSTRACT

In continuation and extension of previous work, purified cell walls of sonically disrupted viable *Pasteurella tularensis* strains SCHU S5 and 38A were evaluated for immunogenic properties in the monkey (*Macaca mulatta*). Subcutaneous vaccination with 12 mg of the larger cell-wall particles (sedimenting at 30,000 x g), either in one site or equally divided among 6 sites, evoked no febrile response, caused few skin lesions, and stimulated a rapid agglutinin response. Protection afforded animals vaccinated with the wall fraction of strain SCHU S5 indicated promise of definition of the sites of the antigens in the cell that are involved in evoking immunity and potential for the preparation of an effective nonviable vaccine.

I. INTRODUCTION

We earlier reported[1] that significant protection of mice* against challenge with highly virulent Pasteurella tularensis SCHU S4 resulted following vaccination with nonviable specific cellular structures of strains SCHU S4, LVS, 38A, and SCHU S4-2 (Table 1). The mouse protection indices, which include in one statistic both percentage of mortality and length of survival time, were calculated according to the method of Meyer and Foster.[2] Significance was attached only to those data deviating more than -6 standard deviations from the means of the nonvaccinated mice so that P is less than 0.001; Meyer and Foster attributed significance to values whose P's are less than 0.02. The 60GP fraction representing the smaller particles of the cell-wall - membrane structure contained the major components capable of eliciting an immune response, although the larger cell-wall fragments (30GIP) derived from strain SCHU S4 also evoked a good protective response. This report is concerned with the immunogenic response * the monkey (Macaca mulatta) of 30GIP fractions prepared from cells of strains SCHU S5 and 38A.

TABLE 1. PROTECTION OF MICE FOLLOWING INOCULATION WITH CELLULAR FRACTIONS FROM SEVERAL STRAINS OF PASTEURELLA TULARENSIS

P. tularensis strain	Mouse Protection Index[a]/ Cell Fraction			
	30GIP	30GIIP	60GP	60GS
SCHU S4	10.1[b]/	13.3	7.9[b]/	11.8
SCHU S4-2	15.9	14.3	11.3[b]/	11.8
LVS	13.7	11.4[b]/	4.4[b]/	12.0
38A	11.7	12.5	9.1[b]/	10.3[b]/

a. LVS-vaccinated mice MPI = 1.1, Nonvaccinated mice MPI = 17.9.
b. Significance P <0.001.

1. Cuss, M.L. 1962. Cell wall studies of Pasteurella tularensis. Bacteriol. Proc. p. 93 (Abstr.)
2. Meyer, K.F., and L.E. Foster. 1948. Measurement of protective serum antibodies in human volunteers inoculated with plague prophylactics. Stanford Med. Bull. 6:75-79.

* In conducting the research reported here, the investigator adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

II. MATERIALS AND METHODS

The 40 monkeys used were grouped by the standard procedure of randomized sampling from a table of 10,000 randomly assorted digits and were vaccinated subcutaneously at either 6 body sites or 1 site. Cell-wall preparations from strains SCHU S5 and 38A were inoculated either in 12-mg amounts in the interscapular region or in 2-mg amounts at each of 6 body sites: the interscapular region, the base of the spinal column, both upper arms, and both thighs. A group of nonvaccinated monkeys and a group vaccinated in the interscapular region with 10^6 viable cells of the live tularemia vaccine (LVS) served as controls. The numbers of monkeys and treatments are summarized in Table 2.

The 30GIP fractions were prepared by sonic disruption of viable cells, differential centrifugation, and washing in phosphate buffer and water, as we have described elsewhere.[3] The preparations had been stored for more than 2 years at 10 to 15 C as a dry powder. Since this material was not wettable in distilled water and formed large clumps that would not pass through a 24-gauge needle, sonication was required to disperse the suspensions. One hr and 40 min was required to prepare the 30GIP fraction of SCHU S5, and 1 hr to prepare the 38A strain at maximum output voltage of a Raytheon 9-kc magnetostrictor oscillator.

3. Gues, M.L., and D.C. Wharton. 1961. Cell wall studies of *Pasteurella tularensis*. Bacteriol. Proc. p. 129 (Abstr.)

TABLE 2. DESIGN FOR EVALUATION OF 30GIP FRACTIONS

	Group 1	2	3	4	5
No. monkeys	9	9	9	8	5
Vaccination	None	10^8 viable LVS interscapular sc	12 mg 30GIP from SCHU S5 interscapular sc	2 mg 30GIP from SCHU S5 in each of 6 sites sc	12 mg 30GIP from 38A interscapular sc
Vaccination response period, days	21	→	→	→	→
Challenge	1200 viable SCHU S4 cells interscapular sc	→	→	→	→
Challenge response period, days	30	→	→	→	→

Observations: temperature daily;
agglutinin titer weekly;
body weight at beginning and end of each period; and
at death or sacrifice gross pathology and isolation of organisms from spleen, liver, lung, and heart blood.

III. RESULTS

After vaccination with any of the fractions the body temperatures of the monkeys remained within the normal range of 100 to 102.5 F. Of the 22 animals vaccinated with cell-wall fractions, only 2 showed minor lesions at the site of inoculation and these healed rapidly. Eight of the 9 LVS-vaccinated monkeys developed lesions that were completely healed by the time of challenge. The mean reciprocal agglutinin titers for each group after vaccination and challenge are shown in Table 3. The SCHU S5 wall fraction stimulates a peak titer 8 to 14 days after inoculation, but the agglutinin response to the live tularemia vaccine continues to rise until the time of challenge. After challenge with 1200 cells of P. tularensis SCHU S4 the titers of the vaccinated groups increase, but not at the rapid rate observed in the nonvaccinated controls. The lesions at the site of challenge were significantly larger and took longer to heal among the wall-vaccinated groups than the LVS-vaccinated and normal challenged groups.

TABLE 3. MEAN RECIPROCAL AGGLUTININ TITERS FOR EACH TEST GROUP FOLLOWING VACCINATION AND CHALLENGE

Group	Day 0	8	14	21	28	35	42	49
Control		0	0	0	3	80	424	1106
LVS viable		40	52	96	176	176	176	274
SCHU S5 cell wall 6 sites	VACCINATION	72	160	71	66	108	280	472
SCHU S5 cell wall 1 site		110	104	55	69	132	208	380
38A cell wall 1 site		50	61	53	61	80	140	296

CHALLENGE[a]

a. Approximately 1200 cells of P. tularensis SCHU S4.

No febrile response was observed after vaccination. Figure 1 shows the temperature responses of 3 groups of monkeys after challenge. The data derived from the 38A cell-wall-vaccinated group was omitted because the febrile response closely approximated that of the normal control group. Similarly, the data on the group vaccinated with SCHU S5 cell walls at 1 site were not included since the curve did not differ from that of the group vaccinated with SCHU S5 cell walls at 6 sites. The febrile response of the LVS-vaccinated group was minimal, the normal controls were maximal, and the SCHU S5 wall-vaccinated group response lay between. However, after the 14th day no difference in temperature elevation was detectable among the 3 groups, although a trend toward higher febrile response may be discernible in the surviving monkeys of the control group.

Table 4 shows the mortality and body weight changes in each group during the experiment. During the test period, 5 of 9 controls died of tularemia in contrast to 2 of 17 SCHU S5 cell-wall-vaccinated monkeys and none of 9 LVS-vaccinated monkeys. One of 5 of the 38A cell-wall-vaccinated monkeys died. Body weight changes were not significant in any of the groups except the 38A cell-wall group, which showed a 20% loss in weight.

TABLE 4. MORTALITY AND BODY WEIGHT CHANGES

Group	Dead/Total	Day of death	Group Body Weight Change g	Group Body Weight Change %
Control	5/9	4,7,9,9,28	+45	+1.8
LVS viable	0/9	-	+160	+6.4
SCHU S5 cell wall, 6 sites	1/8	17	+44	+1.8
SCHU S5 cell wall, 1 site	1/9	10	-44	-1.8
38A cell wall, 1 site	1/5	21	-500	-20.0

Differentiation of the groups for disease severity based on gross pathology at sacrifice 30 days after challenge could not be made with any certainty since all but 2 monkeys had some spleen or lung involvement or both.

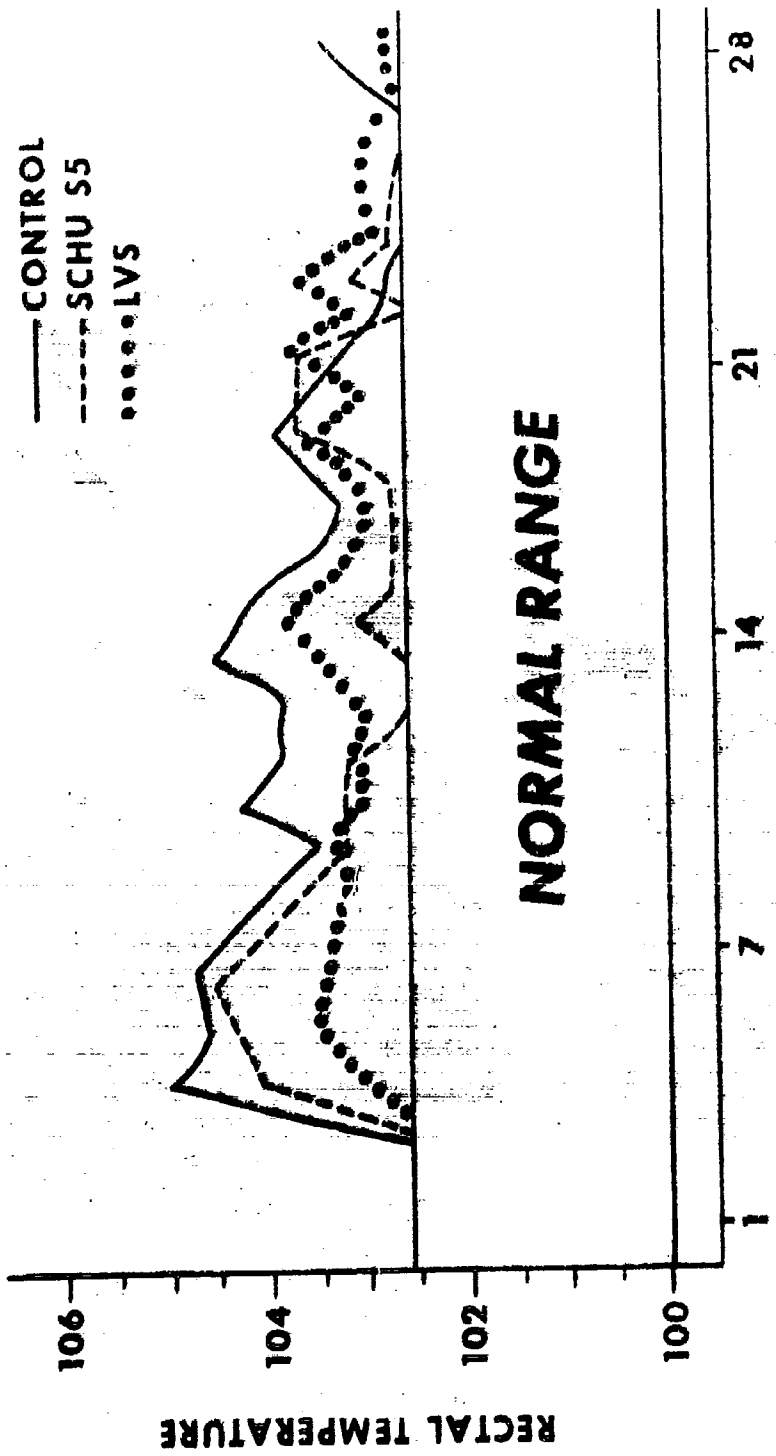

Figure 1. Temperature Response of Vaccinated Macaca mulatta after Challenge with 1200 Cells of Pasteurella tularensis SCHU S4.

The gross pathology of monkeys that died during the test period was indicative of tularemia and was confirmed by cultural and serological identification of the causative organisms from tissues.

Both LVS and SCHU S5 cell-wall-vaccinated monkeys underwent a more benign course of disease than nonvaccinated controls. However, the results do not permit a quantitative comparison of immunogenicity of the different vaccines. The value of this experiment lies in the fact that protection against virulent challenge was evoked by the SCHU S5 cell-wall fraction. These results are sufficient to indicate promise of definition of the sites of the antigens in the cell that are involved in evoking immunity and potential for the preparation of an effective nonviable vaccine. One may also speculate that the 60CP fraction may be more efficacious in the monkey since it stimulated greater protection in the mouse (Table 1) than did the 30CP wall fraction. The questions of method of disruption of the bacterial cells for separation of cellular fractions, the role played by the cytoplasmic membrane, and the size of the particle that best evokes immunity are yet unexplored and need to be resolved so that the fractions prepared are biologically active and structurally as nearly natural as possible.